UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/36
5 February 1979

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE[1]

No. 36

A STUDY ON THE DETERIORATION OF MORPHINE IN THE FRESH LATEX OF PAPAVER SOMNIFERUM L.

Part I

by: V.S. Ramanathan

Chief Chemist (retired)
Central Revenues Control Laboratory
New Delhi-110012
India

1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

GE. 79-10586

INTRODUCTION

Opium is traditionally collected in India by making incisions (lancing) in capsules of Papaver somniferum during the afternoon hours and scraping off the air dried latex (opium) from the capsule surface the next morning. There is an interval of 12 to 16 hours from the time of lancing to the collection of opium. Normally 3 to 4 lancings, each lancing being done at an interval of 72 hours, are carried out to obtain the maximum quantity of latex. The composite of opium of all lancings from each cultivator is transported to the Government factories where it is analysed for morphine content. Normally there is a time lag of more than a month between the collection of opium and the actual estimation of morphine. Atmospheric day temperatures during summer months (March to May) vary from 32°C to 40°C when the opium is exposed to sun and hot air, before and during collection, on farm storage and transport to the factory. These factors may result in the deterioration of morphine in fresh latex.

As early as 1935, Troshohenko et al. (1) pointed out that the fresh latex, immediately after lancing, contained 20% morphine on dry basis compared to 10 to 13% in opium following collection and storage. Fulton (2) in his publication on "The Opium Poppy and Other Poppies" has also reported similar findings. According to Bentley (3), the fall of morphine in fresh latex from 21.8 to 12% is due to atmospheric oxidation. Ramstad (4) has observed that 50% of morphine present in opium undergoes deterioration due to the peroxidase enzyme action. He stated that the morphine content deteriorated from 22% to 11% to 13% in his studies. Results on the deterioration of morphine in fresh latex of Papaver somniferum have been published by Professor Dalev et al. (5).

The present study aims to ascertain whether the fresh latex collected from capsules of Indian opium poppy actually contains 20% morphine and the percentage loss in morphine content during the 24 hours following lancing. Many preservative chemicals, electrolytes, antioxidants and enzymatic deactivators were tested to determine their potential for arresting the deterioration of morphine in fresh latex and findings are reported in this paper.

MATERIALS AND METHODS

In November 1974 the experimental field of the Government Opium and Alkaloid Works, Neemuch, India, was sown with seed of the poppy varieties Telia (Indian) and Iranian. Lancing of capsules commenced in March 1975. Capsules were lanced during the afternoon and fresh latex collected from their surface one hour after lancing.

The one hour period was necessary to accumulate sufficient latex for the various tests. About 500 to 600 capsules were lanced and latex collected. Morphine content in fresh latex was estimated in the field using a mobile laboratory. A sample of the freshly collected latex was kept as a check under laboratory conditions for 24 hours after which its morphine content was determined.

Fresh latex collected in the field was divided into samples of 8 grammes each. 0.5 gramme of one of the 24 preservative chemicals and control listed below was added to and thoroughly mixed with a sample of the fresh latex. The samples with preservatives and the control were analysed for morphine content the next day.

The fresh latex oozings on the surface of the capsules after one hour following lancing were collected the next morning before 8 a.m. and morphine content estimated.

Morphine percent in fresh latex, latex with added preservatives and oozings was estimated separately for each of the three lancings.

Preservative chemicals investigated to arrest deterioration of morphine in fresh latex include: (1) sodium ascorbate, (2) hydroxylamine hydrochloride, (3) butylated hydroxy toluene, (4) Kalanamak[2/], (5) syrupy phosphoric acid, (6) aniline, (7) potassium fluoride, (8) citric acid, (9) powdered yeast, (10) borax, (11) sodium phosphate, (12) sodium carbonate plus sodium bicarbonate (1:1), (13) formalin, (14) phenol, (15) lead acetate, (16) sodium sulphate, (17) sodium acetate, (18) sodium bicarbonate, (19) calcium chloride, (20) gallic acid, (21) pyrogallic acid, (22) sodium chloride (common salt), (23) sodium carbonate, (24) celin (ascorbic acid) and (25) distilled water.

In two separate experiments, the surface of poppy capsules in the field were smeared with a cotton swab of 5% dispersion of sodium ascorbate in glycerine and 5% aqueous sodium sulphite before lancing. The lancing knives were dipped in the test solutions immediately before lancing the capsules. The glycerine was not satisfactory as a solvent since the fresh latex would not form pellicles and stick to the surface of capsules. The exudate dropped from the capsules on to the leaves below. Also, glycerine contaminated the latex, thereby making accurate estimation of morphine in opium difficult.

2/ A local product composed of sodium carbonate, sodium chloride, sodium sulphide and sodium sulphate.

In addition to tests in the field of the Government Opium and Alkaloid Works, Neemuch, experiments with selected preservatives were evaluated under actual field conditions at 3 selected farms.

Method of estimation of morphine:

Determination of morphine in the fresh latex and opium samples with preservative chemicals was based on the method laid down in the British Pharmacopoeia, 1963 (6).

pH of the fresh latex:

pH of fresh latex immediately after lancing was estimated at intervals of 30 minutes for a period of 2 hours. Initial and final readings of pH were compared to examine the possible bio-chemical reactions taking place in the latex.

RESULTS AND DISCUSSION

Results obtained in 13 field experiments on the deterioration of morphine and effects of preservatives in the fresh latex are given in Tables 1 to 5.

Fresh latex from the first lancing contained the highest morphine percent. The variety Telia had the highest morphine content from 14.50% to 16.46% for the varieties tested (Table 1). In all varieties, there was a decrease in morphine content between the fresh latex and oozings collected the next morning. The maximum loss of 5.89 units was observed in the Iranian variety. Oozings showed a morphine content as low as 9.47% compared to the much higher values obtained with fresh latex (Table 1).

There was a definite lowering of morphine percent between the fresh latex of first lancing and that obtained from subsequent lancings (Table 1) (11.52 to 12 % for the second lancing and 9.30 to 10.86 % for the third lancing). The oozings of second lancing had the lowest morphine percent, only 8.62%. Reductions in morphine content in the opium checks stored for 24 hours under laboratory conditions was also observed when compared to values obtained in fresh latex. The observations were similar in all varieties tested. The maximum decrease occurred with the Iranian variety (Table 1).

The effects of preservative chemicals on morphine content in fresh latex of Iranian and Telia varieties are given in Tables 2 and 3. Morphine content in fresh latex of Telia varied from 16.22% to 16.70% for the first lancings. Out of 24 chemicals tested, only phosphoric acid, Na_2CO_3+ $NaHCO_3$ (1:1) and phenol showed preservative action. In the latex of second lancing, positive preservative effects are indicated with the addition of phosphoric acid, sodium acetate, sodium chloride and citric acid (Table 3).

For the Iranian variety, sodium ascorbate and hydroxylamine hydrochloride indicated preservation of morphine even in latex from the third lancing (Table 2).

Results obtained from the three selected farmers are given in Table 4. Of the ten chemicals tested, preservative effects were observed only from Kalanamak, phosphoric acid, hydroxylamine hydrochloride, Na_2CO_3+$NaHCO_3$, sodium chloride and phenol.

In experiments with chemicals smeared on the surface of capsules with a swab before lancing, loss in morphine could not be arrested with sodium ascorbate and sodium sulphite solutions. Smearing each capsule with a preservative is a cumbersome operation under field conditions. Morphine estimation on dry matter basis was found difficult where sodium ascorbate was smeared since the latex collected was contaminated with glycerine.

CONCLUSION

The fresh latex collected one hour following lancing has 16.7% morphine for Indian variety Telia. This was the highest morphine content recorded for five varieties in the study. Morphine content in latex exposed to laboratory conditions for 24 hours was lowered by 2.48 units. There was a decrease of 5.8 units in morphine in oozings, indicating the effects of sun and heat following lancing on the deterioration of morphine.

The fresh latex collected had a pH of 6.8 which decreased to 5.0 after two hours. The lower pH indicates the biochemical reactions taking place in the latex when exposed to atmospheric conditions. Reduction in morphine content could be attributed either to its decomposition or partial conversion into other organic compounds.

Of the 24 chemicals used to prevent the deterioration of morphine, six were promising, namely, Kalanamak, sodium chloride, citric acid, sodium acetate, sodium ascorbate and hydroxylamine hydrochloride. Kalanamak is used as a preservative for pickles and has medicinal properties. Sodium sulphide, a constituent of Kalanamak, is a reducing agent and an enzyme poison. Sodium carbonate neutralises the acids produced in the latex and sodium chloride, a strong electrolyte, is an inhibitor of ensymatic action.

Studies with preservatives are being continued to arrive at definite conclusions on their effects in arresting the deterioration of morphine in the latex of Papaver somniferum.

ACKNOWLEDGEMENTS

This study was financially supported by UNFDAC and the author is grateful to Dr. O.J. Braenden, Director, U.N. Narcotics Laboratory, Geneva, for the keen interest evinced in the work. The author is also thankful to the officers and the staff in the Narcotics Department, Government of India, Ministry of Finance and Chemists in the Government Opium Factory, Neemuch for all the help rendered to him during the study.

REFERENCES

(1) Vorozhtsov, N.N. and Troshchenko, A.T. "Der Morphingehalt in Milchsafte des Opium Mohns" Comptes Rendus de l'Académie des Sciences de l'Union des Républiques socialistes soviétiques 1935, (2) 555-558 (In Russian; German Summary 558); Nilov, V.I., Nilova W.P. and Troshchenko, A.T., "Fermentative Oxidation of Morphine in the latex of the Opium Poppy" Biokhimiyal, 165-82 (1936); Chemical Abstracts 30,7779.

(2) Fulton, C.C. "The Opium Poppy and other Poppies", U.S. Treasury Department, Bureau of Narcotics, Washington D.C., 1944, 29.

(3) Bentley, K.W., "The Chemistry of the Morphine Alkaloids" Clarendon Press, Oxford, 1954, 15.

(4) Ramstad, E., Modern Pharmacognosy, McGraw Hill Book Company Inc., New York, 1959, 343, 344, 407.

(5) Dalev, D., et al., Bulletin on Narcotics, XII, (1) (1960), 25.

(6) British Pharmacopoeia, 1963, 544.

TABLE 1

MORPHINE CONTENT IN FRESH LATEX AND OPIUM

Location:	Opium Factory Neemuch	Opium Factory Neemuch	Laxmipura (Rajasthan)	Bharukheda (M.P.)	Khadavda (M.P.)	Opium Factory Neemuch	Opium Factory Neemuch	Opium Factory Neemuch	Opium Factory Neemuch
Variety:	Iranian	Telia	Dhaturia	Ranjhatak	Kanta Telia	Telia	Iranian	Telia	Iranian
Temperature °C									
(i) field morning 7 a.m.	20	20	21	20	20	20	20	20	20
(ii) field midday	33	33	33	33	34	34	34	34	34
(iii) laboratory midday	28	28	27	30	29	30	30	28	28
Relative humidity: %	48	48	43	44	44	43	43	44	44
Date of lancing:	10.3.75	10.3.75	13.3.75	14.3.75	15.3.75	11.3.75	12.3.75	13.3.75	14.3.75
Number of lancing:	First					Second		Third	
	Anhydrous Morphine on dry matter					Anhydrous morphine on dry matter		Anhydrous morphine on dry matter	
	%	%	%	%	%	%	%	%	%
Fresh latex analysed immediately	16.43	16.46	16.40	14.50	15.50	11.52	12.00	10.86	9.30
Latex analysed after keeping in the laboratory for 24 hrs.	13.95	15.68	15.30	13.69	-	9.87	11.36	10.41	8.83
Oozings, collected next morning and analysed the same day.	10.60	14.79	10.99	9.47	13.25	9.49	8.62	-	-

TABLE 2

EFFECT OF PRESERVATIVES ON MORPHINE CONTENT

Location: Field in Government Opium Factory, Neemuch (M.P.)

Variety: Iranian

Temperature °C			
(i) Field	33	33	34
(ii) Laboratory	28	28	28
Date of lancing:	10.3.75	12.3.75	14.3.75
Number of lancing:			

Treatments	Morphine content on dry matter basis		
	%	%	%
Opium without preservative as check sample	13.95	11.36	8.83
Opium + 0.5g hydroxylamine hydrochloride + 1 ml water	14.61	-	8.72
Opium + 0.5g of sodium ascorbate + 1 ml water	14.21	-	9.13
Opium + 0.5g of $CaCl_2$ + 1 ml water	-	10.55	-
Opium + 0.5g of KF + 1 ml water	-	-	8.72
Opium + 0.5g gallic acid	-	-	8.77
Fresh latex analysed immediately	16.43	12.0	9.30

TABLE 3

EFFECT OF PRESERVATIVES ON MORPHINE CONTENT

Location: Field in Government Opium Factory, Neemuch (M.P.)

Variety: Telia

Temperature Field °C 33-34

Temperature Laboratory °C 28-30

Date of lancing:	10.3.75	11.3.75	13.3.75	15.3.75
Number of lancing:	First		Second	Third
Treatments	Morphine percent on dry matter			
1. Opium without preservative as check sample	15.93	15.43	9.87	10.41
2. Opium + 0.5g B.H.T.	12.26	-	-	-
3. Opium + 0.5g Kalanamak	15.25	-	-	-
4. Opium + 11 drops phosphoric acid	16.06	-	11.70	-
5. Opium + 11 drops aniline	13.64	-	-	-
6. Opium + 0.5g KF	15.09	-	-	-
7. Opium + 0.5g citric acid + 1 ml water	15.31	-	11.25	-
8. Opium + 0.5g yeast	13.86	-	-	-
9. Opium + 0.5g borax	12.50	-	-	-
10. Opium + 0.5g sodium phosphate	-	8.59	-	-
11. Opium + 0.5g Na_2CO_3 + $NaHCO_3$ (1:1)	-	16.15	-	-
12. Opium + formalin (30%) 25 drops	-	12.60	-	-
13. Opium + 0.5g ml phenol	-	15.91	-	-
14. Opium + 0.5g lead acetate + 1 ml water	-	14.94	-	-
15. Opium + 0.5g sodium sulphite + 1 ml water	-	14.06	-	-
16. Opium + 0.5g sodium acetate + 1 ml water	-	15.03	11.57	-
17. Opium + 0.5g $NaHCO_3$ + 1 ml water	-	14.55	-	-
18. Opium + 0.5g gallic acid	-	-	7.86	-
19. Opium + 0.5g pyrogallic acid	-	-	7.57	-
20. Opium + 0.5g NaCl + 1 ml water	-	-	11.57	-
21. Opium + 0.5g Na_2CO_3+ 1 ml water	-	-	10.69	-
22. Fresh opium latex analysed immediately on collection	16.70	16.22	11.52	10.86

TABLE 4

EFFECT OF PRESERVATIVES ON MORPHINE CONTENT

Name of village and State:	Lakshmipura (Rajasthan)	Bharukheda (Madhya Pradesh)	Khadavda (M.P.)
Variety:	Dhaturia	Ranjhatak	Kanta Telia
Temperature Field °C	33	33	34
Temperature Laboratory °C	27	30	29
Date of lancing:	13.3.75	14.3.75	15.3.75
Treatments	Morphine percent on dry matter		
1. Opium + 11 drops H_3PO_4	15.58	-	14.43
2. Opium + 0.5g citric acid	14.96	-	14.69
3. Opium + 0.5g Kalanamak	16.39	-	15.19
4. Opium + 0.5g hydroxylamine hydrochloride	14.96	-	-
5. Opium + 0.5g Na_2CO_3 + $NaHCO_3$ 1:1 + 1 ml water	-	14.31	-
6. Opium + 11 drops phenol	-	14.26	-
7. Opium + 0.5g lead acetate + 1 ml water	-	13.36	-
8. Opium + 0.5g sodium acetate	-	13.89	14.21
9. Opium + 0.5g celin (Vitamin C)	-	-	14.85
10. Opium + 0.5g NaCl	-	-	15.25
11. Opium + 60 ml distilled water	-	-	15.11
12. Opium without preservative as check sample	15.30	13.69	-
13. Fresh opium latex analysed immediately after collection	16.40	14.50	15.50

TABLE 5

PRESERVATIVE EFFECT OF SODIUM ASCORBATE AND SODIUM SULPHITE

Location: Government Opium Factory, Neemuch (M.P.)

Variety: Telia

Temperature °C

(i) Field 33.5

(ii) Laboratory 29.0

Relative humidity:

Field percent 43

Date of lancing: 12.3.75 13.3.75

Number of lancing: First First

Anhydrous morphine percent on dry matter

Treatments	Capsules smeared with 5% dispersion of		
	Sodium ascorbate	Sodium sulphite	Check
1. Fresh latex	16.14	11.46	16.46
2. Opium without preservative kept for 24 hrs	-	11.66	15.68
3. Opium + 0.5g sodium ascorbate	15.00	-	-
4. Oozings	8.83	10.28	14.79

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/37
5 February 1979

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE[1/]

No. 37

SIMULTANEOUS IDENTIFICATION OF MAJOR ALKALOIDS IN POPPY PLANTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by: Güneş Batu, Suat Ungan, Okan Tarhan

Middle East Technical University
Chemistry Department
Ankara, Turkey

1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

GE. 79-10583

INTRODUCTION

Several chromatographic methods have been reported for quantitative analysis of alkaloids in poppy plants or gum opium using either the isocratic or the gradient elution techniques (1-15). The use of an isocratic method is not practical due to the fact that one has to use two or more different solvent mixtures as the mobile phase for the complete analysis. Also, in this method the chromatographic column has to be cleaned or regenerated after several runs in order to obtain reproducible results.

The objective of this work is to present a rapid and sensitive method by which the major alkaloids present can be analyzed in one run, and therefore routine and automated repetitive analyses are possible.

METHOD

1. Extraction

0.2-0.5 g samples ground to Tyler -48 mesh size were weighed and placed in a 20 ml volumetric flask. 10 ml of 4% H_2SO_4 solution were added together with 2-3 mg brucine (dihydrate) which served as the internal standard. The contents of the flask were shaken for one hour. A clear extract was obtained by centrifugation and placed in a 50 ml separatory funnel. An equal volume of hexane was added to the filtrate, the mixture was shaken and the organic phase was discarded. 25% ammonia solution was added to the aqueous phase to adjust the pH to 10-11. Alkaloids were then extracted with an equal volume of organic phase consisting of chloroform and iso-propanol (3:1).

2. Apparatus

A Waters Assoc. Model ALC/GPC 204 liquid chromatograph equipped with a second Model 6000A solvent delivery system. Model 660 programmer and Model U6K universal injector were used in these studies. μ Bondapak C_{18} (4mm ID X 30 cm) column was selected because it provided high column efficiency and excellent reproducibility. Alkaloids were determined at 280 nm using a UV detector.

3. Reagents

All reagents were obtained from Merck A.G. and were of analytical grade. Alkaloid standards were obtained from T.M.O. (The Turkish Soil Products Office) and Knoll A.G.

4. Solvent systems

(a) Polar solvent: 1% (w/v) $(NH_4)_2CO_3$ solution in water;

(b) Less polar solvent: methanol.

5. Conditions

Gradient elution from 55% to 100% methanol in 15 minutes by curve No. 9. Initial conditions were attained in 5 minutes by reversing the programme using curve No. 6 after each analysis. The flow rate of the mobile phase was kept constant at 2 ml/min.

6. Calculations

Brucine (dihydrate) was used as the internal standard. K values for the alkaloids calculated from equation [1] are given in Table I with their respective standard deviations.

$$K = \frac{W}{W_B} \cdot \frac{A_B}{A} \qquad [1]$$

where:

W = weight of an alkaloid in standard solution,

W_B = weight of brucine (dihydrate) in standard solution,

A = peak area of an alkaloid,

A_B = peak area of brucine (dihydrate).

The alkaloid content of an unknown sample was calculated according to equation [2] given below:

$$W\ (\%) = K \frac{A}{A_B} \frac{W_B}{W_S} \cdot 100 \qquad [2]$$

where:

W_S = weight of the unknown sample.

TABLE I

K values of alkaloids with respect to brucine (dihydrate)

Compound	K (as in equation [1])*
Morphine	3.75 ± 0.07
Codeine	3.78 ± 0.05
Thebaine	0.72 ± 0.02

* Valid only with the chromatographic conditions mentioned above.

RESULTS AND DISCUSSION

The results of the method developed are in a good agreement with the results for reference samples provided by the U.N. Narcotics Laboratory (Table II).

TABLE II

Results of analysis of several poppy samples

Sample	Extraction method	% Morphine	% Codeine	% Thebaine
UN Sample I	%5 HOAc	1,71*	0,065	-
UN Sample I	%2 H_2SO_4	1,73*	-	-
UN Sample I	%4 H_2SO_4	1,77	0,014	0,010
Turkish sample Iznik	%4 H_2SO_4	0,52	-	-

* Private communication - U.N. Narcotics Laboratory, Geneva.

The analysis of the same poppy samples was repeated by thin-layer and gas chromatographic techniques in our laboratory. We believe that the HPLC method proves to be more reliable and less time consuming than the above mentioned methods. Among the various extraction methods described in the literature, we found that 4% H_2SO_4 led to the highest alkaloid content. In order to avoid time loss required for the complete filtration of the mixture, brucine is added into the extraction mixture and the first 5 ml of the filtrate analyzed. Any organic moiety, other than the alkaloids, which may contaminate the HPLC columns is removed by hexane. Brucine with a similar pK_a value as morphine (16) is assumed to be fully protonated in the presence of 4% H_2SO_4 and hence remains in the aqueous phase.

The K values were calculated both using the peak heights and areas. The latter gave much less deviation.

The claimed decomposition of thebaine in acidic medium was checked (17) and no such decomposition was observed in 4% H_2SO_4 for one hour.

The chromatogramme of the test mixture, morphine, codeine and thebaine with brucine (dihydrate) is shown in Figure 1. The chromatogrammes of the U.N. poppy sample and the Turkish poppy sample are given in Figure 2 and Figure 3, respectively.

ACKNOWLEDGEMENTS

The authors are thankful to Turkish Research Council for their financial support and to the U.N. Narcotics Laboratory, Geneva, for supplying the reference samples.

REFERENCES

1. H.A. Lloyd, M.H. Fales, P.F. Highet, W.J.A. van den Heuvel and W.C. Wildman, J. Amer. Chem. Soc., 82, 3791 (1960)
2. S. Yamaguchi, I. Seki, S. Okuda and K. Tsuda, Chem. Pharm. Bull., 10, 755 (1962)
3. K.D. Parker, C.F. Fontan and P.L. Kirk, Anal. Chem., 35, 356 (1963)
4. A. Bechtel, Chromatographia, 5, 404 (1972)
5. J.H. Knox and J. Jurand, J. Chromatogr., 82, 398 (1973)
6. ibid., 87, 95 (1973)
7. D.W. Smith, T.H. Beasley, Sr., R.L. Charles and H.W. Ziegler, J. Pharm. Sci., 62, 1691 (1973)
8. J.D. Wittwer, J. Forensic Sci., 18, 138 (1973)
9. C.Y. Wu, S. Siggia, T. Robinson and R.D. Waskiewicz, Anal. Chim. Acta, 63, 393 (1973)
10. T.H. Beasley, D.W. Smith, H.W. Ziegler and R.L. Charles, J. AOAC, 57, 85 (1974)
11. D. Furmanec, J. Chromatogr., 89, 76 (1974)
12. R. Verpoorte and A.B. Svendsen, J. Chromatogr., 100, 227 (1974)
13. ibid., 100, 231 (1974)
14. E. Murgia and H.F. Walton, J. Chromatogr., 104, 417 (1975)
15. P.J. Twitchett, J. Chromatogr., 104, 205 (1975)
16. Handbook of Chemistry, CRC, 52. ed. D-117
17. H.W. Ziegler, T.H. Beasley, Sr. and D.W. Smith, J. AOAC, 58, 885 (1975)

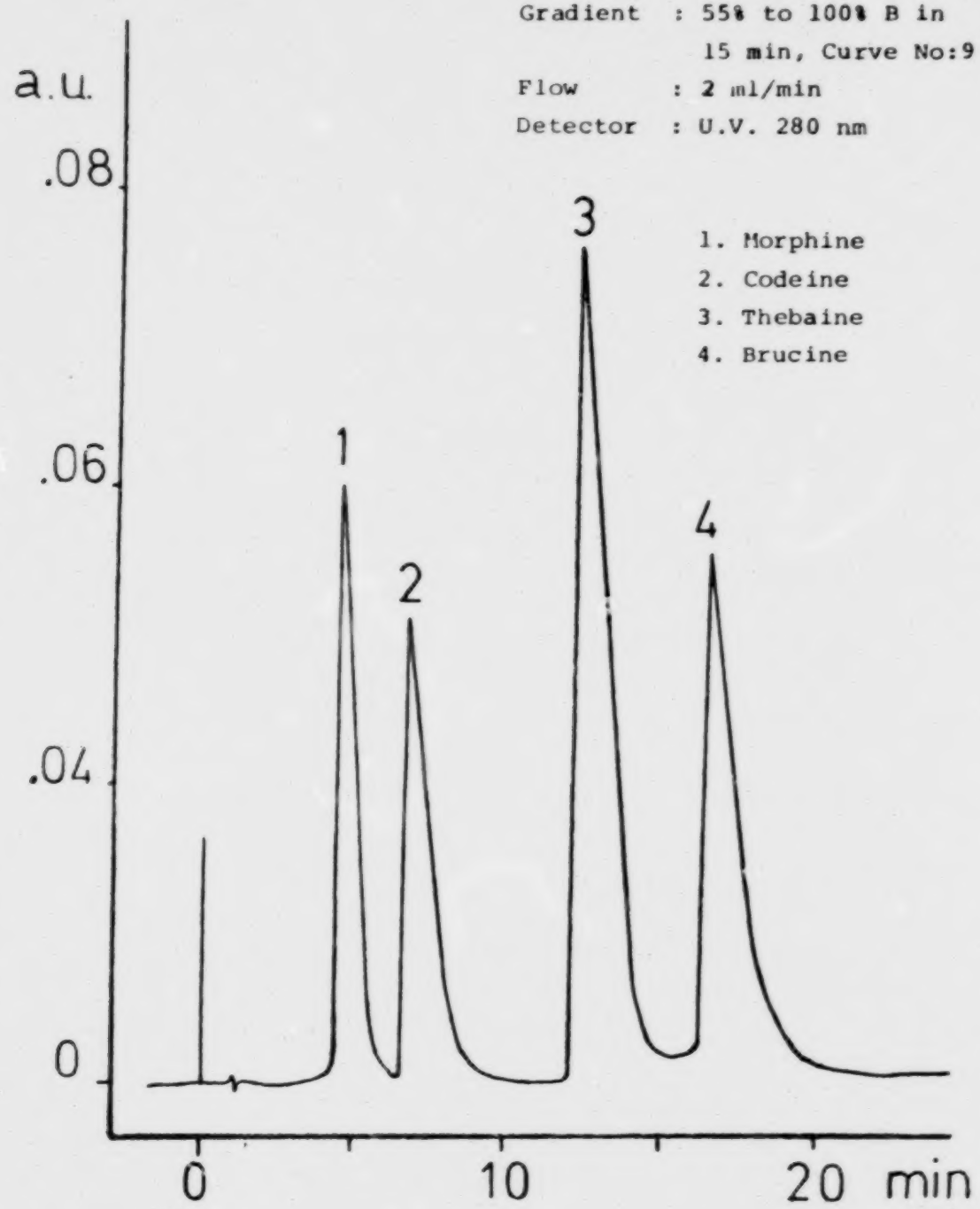


Figure 1 - Chromatogramme of the test sample.

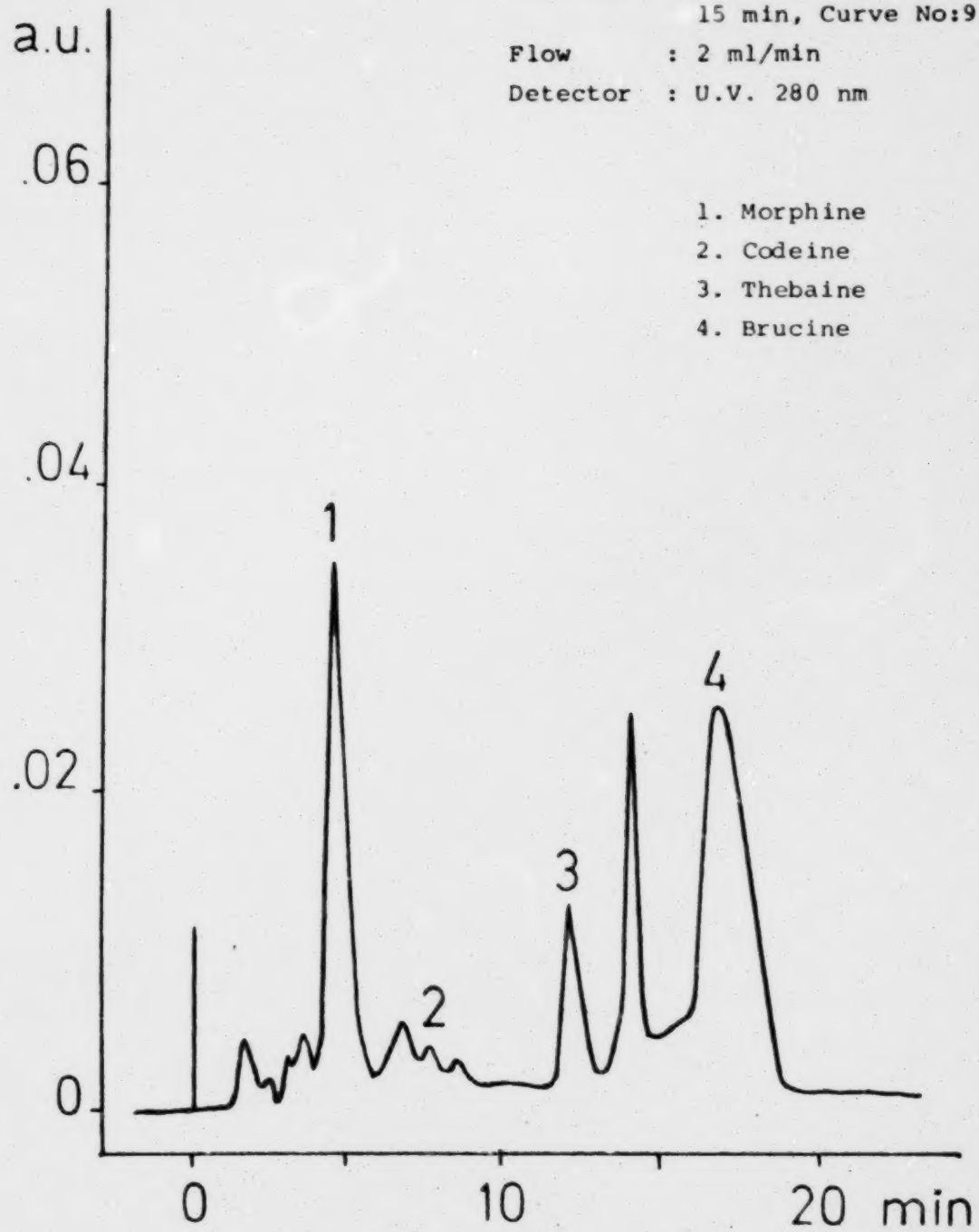


Figure 2 - Chromatogramme of UN sample I.

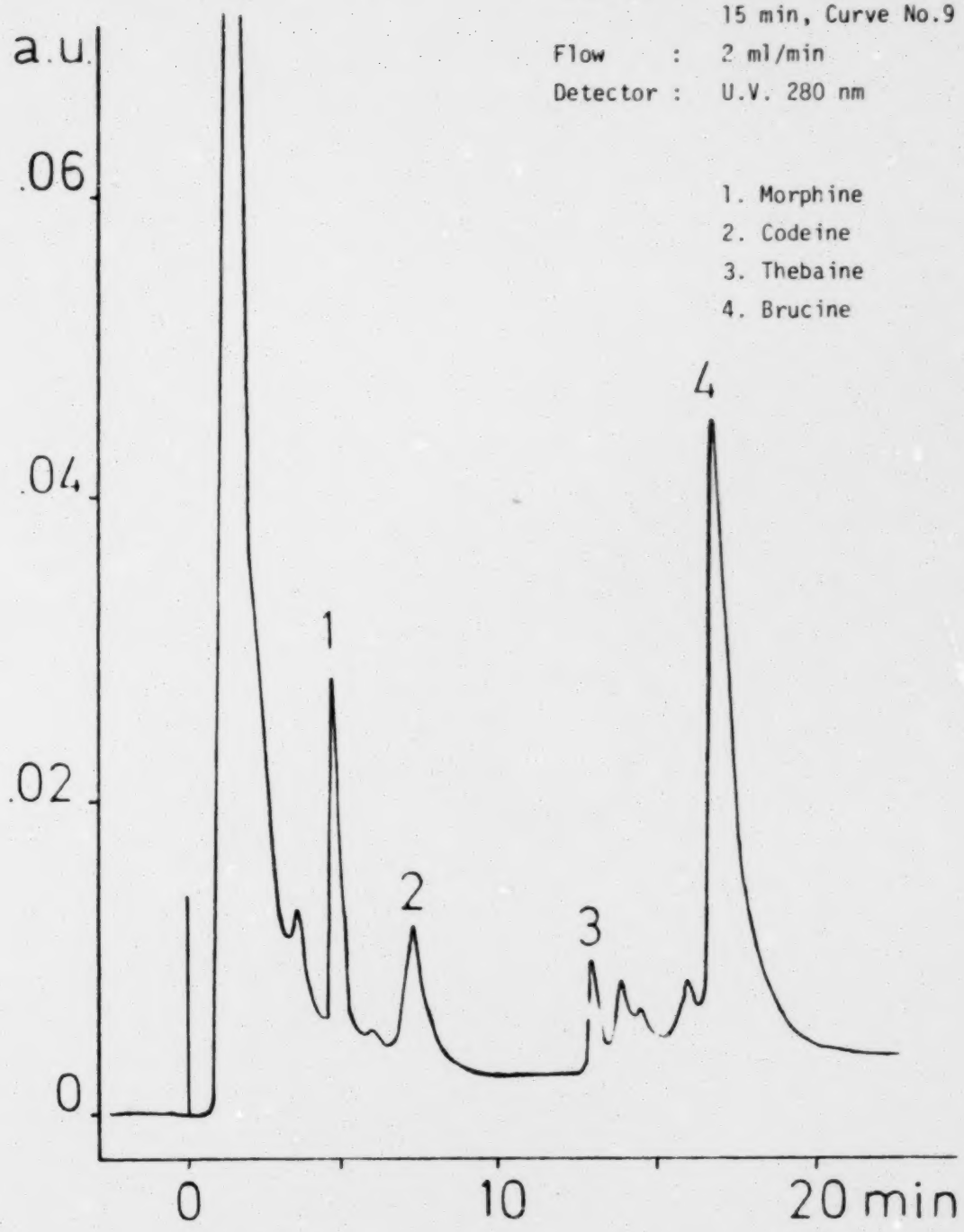


Figure 3 - Chromatogramme of Turkish poppy sample.

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/38
10 April 1979

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE 1/

No. 38

LOSSES OCCURRING IN THE STORAGE OF POPPY (PAPAVER SOMNIFERUM) STRAW

by: Octavio Aguar Monterde

Board of Health
General Mola 54
Madrid, Spain.

1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

GE. 79-11950

INTRODUCTION

Deterioration in stored products frequently occurs without detection, and the extent of loss in quality is seldom realized. The deterioration may begin before harvesting, when the plants are ripening. Bacteria, fungi, insects, rodents, etc., may attack various parts of the plant. Also, quality may be affected by the methods of harvesting, separation and handling, while storage conditions may induce losses by micro-organisms and chemical changes in the products.

Stages at which losses occur

Losses can take place at any of the different stages through which poppy straw passes on its journey from the fields to the extraction plant. It undergoes deterioration during growth; while awaiting harvesting, and after it has been harvested; in transport vehicles in which it is; and in the warehouse during handling and storage (separation of the seeds, preparation of compressed bales of straw, etc.)

The purpose of this study was to determine whether losses in weight and in alkaloids occur after the straw has been collected and processed for storage. Losses caused by methods of harvesting, transport, etc. will be the subject of other investigations.

Losses in weight

Losses in weight of capsules result from the evaporation of moisture and the quantity destroyed by insects, diseases and rodents, and by spillage during transport and storage. Losses from drying of capsules following harvest vary from 10% to 40% depending on their stage of maturity, weather conditions and method of harvest, i.e. hand picking vs combining.

Losses in the stored products caused by rodents are of two main kinds:

(a) Once the capsules have been picked, rodents can consume huge quantities of the seeds, although not of the straw. If the capsules have been picked when they are whole and still contain seeds, the rodents make holes in them to reach and eat the seed.

(b) When the straw has been separated from the seed, the usual practice is to store the latter in jute sacks. These can be burrowed into by rodents, causing enormous losses. Consequently,

it is recommended that the seeds should be stored in bulk after cleaning. This provides less shelter for the rodents.

Losses in alkaloids

It is logical to assume that the phenanthrene alkaloids in the stored poppy straw will deteriorate in the course of time through the action of such factors as humidity, atmospheric oxygen, temperature, method of processing to reduce bulk, etc.

After the poppy straw has been separated from the seed, it is desirable to process it by some method to reduce its bulk. Only "mini-packing" and granulation of capsules will be considered in this paper in reference to their effect on the degradation of alkaloids. These are the two aspects we shall try to study with a view to establishing methods of operation calculated to produce as little loss as possible.

In order to determine the quantitative losses of alkaloids, the following plan of work was adopted:

One kg of capsules without seed was taken from each of two varieties: Papaver somniferum variety celtibericum and a control variety of Papaver somniferum UNS 37. In turn, each sample was divided into two sub-samples and identified as A and B.

Sample A - The capsules were pulverized and divided into three parts, each of which was kept under different conditions: A-1, ambient conditions; A-2, at 4°C; A-3, inert atmosphere and room temperature.

The samples were further divided into five parts for analysis at specific time intervals.

Sample B - Each whole capsule was divided into three parts so that the sub-samples would be similar. Each of the three parts was stored under one of the following conditions after being divided into five parts for analysis at specific time intervals. B-1, ambient conditions; B-2, at 4°C; B-3, inert atmosphere and room temperature.

RESULTS

Results of analyses made on the several dates are given in table 1, and shown in graph form in the attached figures.

With regard to the possible losses of alkaloids resulting from a reduction in the volume of straw, the morphine content of the straw before and immediately after handling was determined by means of two different processes: granulation and mini-packing.

	Granulation		Mini-packs	
	Before	After	Before	After
Morphine	0.368 % 2/	0.366 %	0.42 %	0.418 %

TABLE 1

	Morphine content				
	April	June	August	October	Total losses
Mini-packing	0.42	0.41	0.42	0.39	0.03
Granulation	0.36	0.35	0.34	0.35	0.01

CONCLUSIONS

After reviewing the results of the analyses, we can confirm that there is a loss in morphine content in every case (see figures at the end of the paper).

It would seem that the best method of storage is to keep the straw in as coarse a state as possible.

Any method of handling involving finer grinding will inevitably increase the loss of alkaloids.

Very little degradation of the alkaloid content takes place with the methods we have been using to reduce the volume of the straw.

2/ Figures are the mean for three analyses of each sample. To ascertain whether there were variations in the amount of alkaloids lost during the storage of these two types of compressed product they were kept in storage and analysed at regular intervals (see Table II).

TABLE II

Morphine content of "mini-packed" and ground opium poppy capsules stored under three conditions and analyzed over an eight-months' period

	Date of analyses and morphine content as a % of dry weight				
	31.1.77	2.5.77	4.7.77	5.9.77	Loss of alkaloids
	%	%	%	%	
P. somniferum var. celtibericum					
Powder at room temperature	2.00	1.90	1.94	1.89	0.11
Powder at 4°C	1.84	1.82	1.84	1.79	0.05
Powder in CO_2	1.94	1.93	1.83	1.85	0.09
Capsule at room temperature	1.82	1.82	1.82	1.83	----
Capsule at 4°C	1.82	1.82	1.79	1.75	0.07
Capsule in CO_2	1.82	1.81	1.83	1.83	----
P. somniferum - UMS 37					
Powder at room temperature	0.43	0.40	0.40	0.40	0.03
Powder at 4°C	0.41	0.40	0.39	0.39	0.02
Powder in CO_2	0.42	0.39	0.39	0.39	0.04
Capsule at room temperature	0.40	0.37	0.37	0.38	0.02
Capsule at 4°C	0.41	0.40	0.41	0.39	0.02
Capsule in CO_2	0.43	0.41	0.37	0.37	0.06

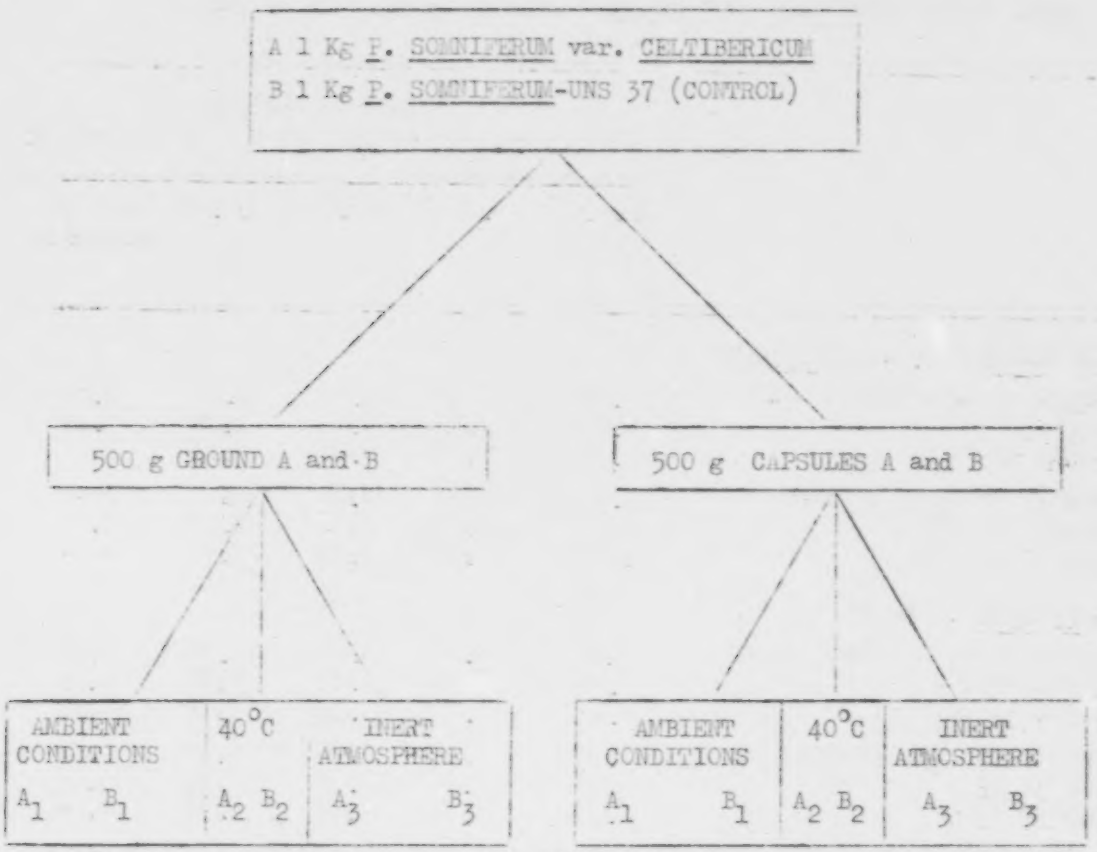


Figure 1 - Diagram of research with opium poppy straw to determine the effect of pre-storage processing and storage environment on phenanthrene alkaloid content.

Figure 2 - Changes in morphine content of P. somniferum var. celtibericum when capsules are pulverized and stored in different environments.

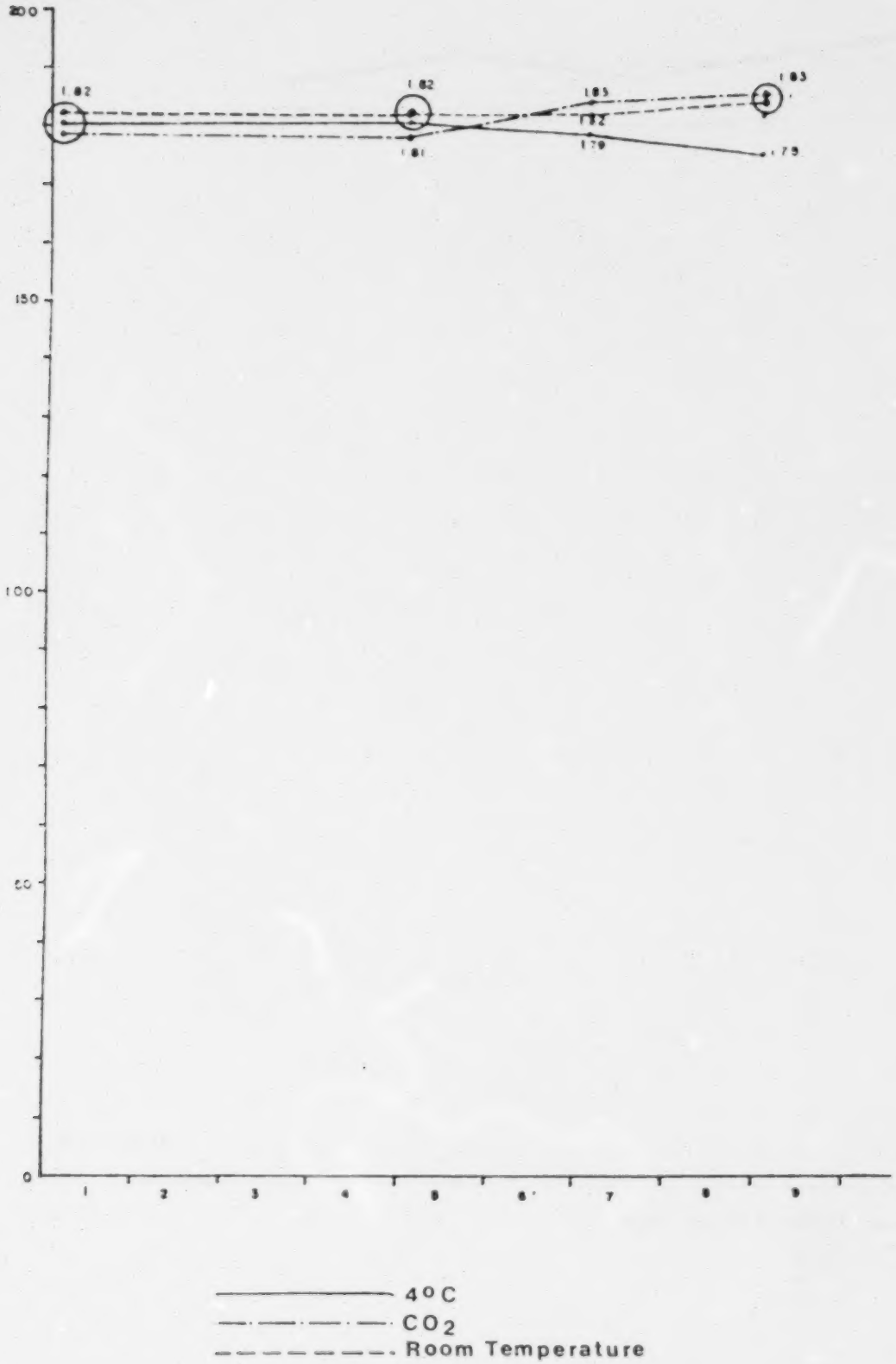


Figure 3 - Changes in morphine content of P. somniferum var. celtibericum when capsules are compacted as "mini-pack" and stored in different environments.

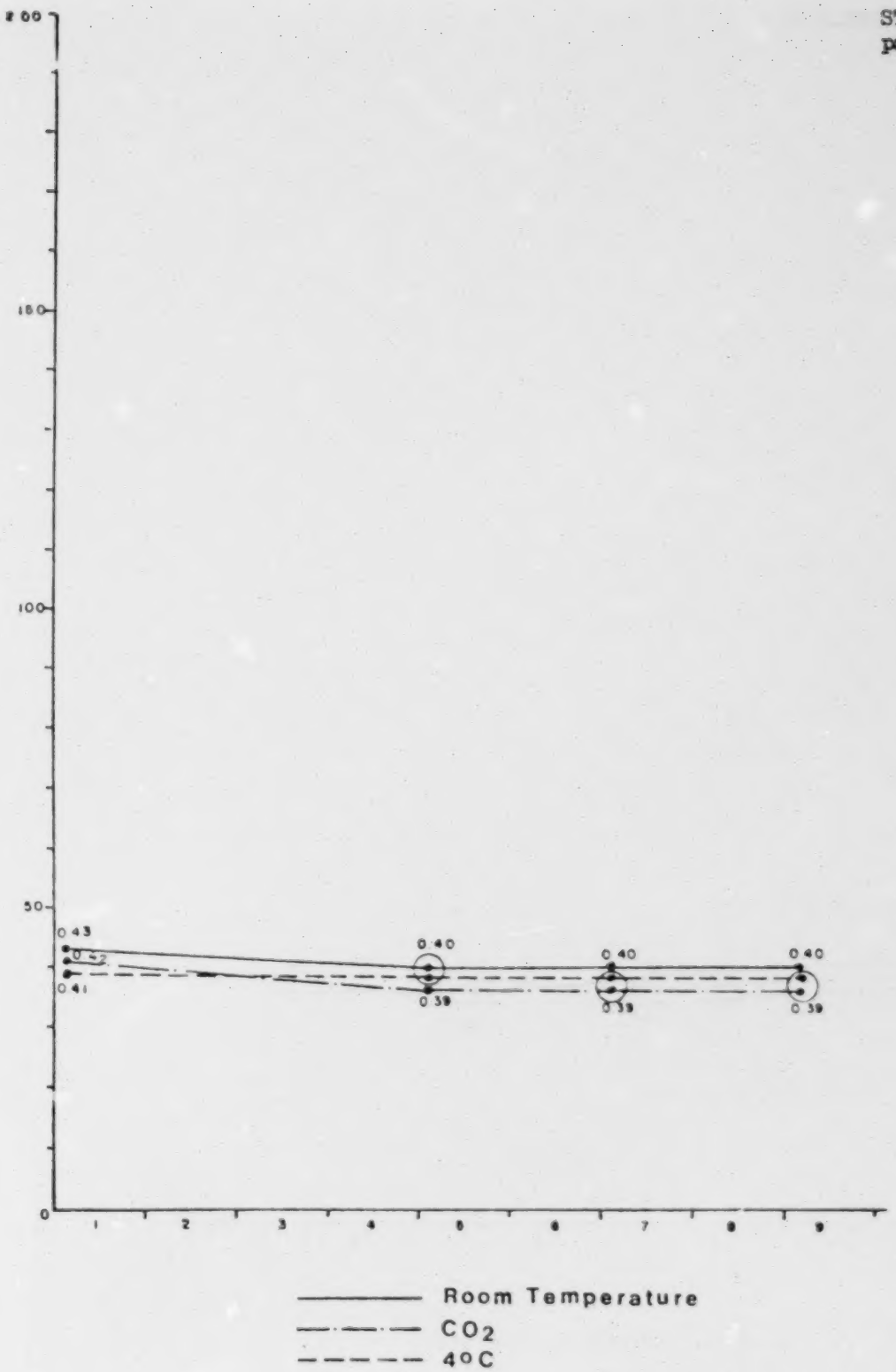


Figure 4 - Changes in morphine content of P. somniferum - UNS 37 when capsules are pulverized and stored in different environments.

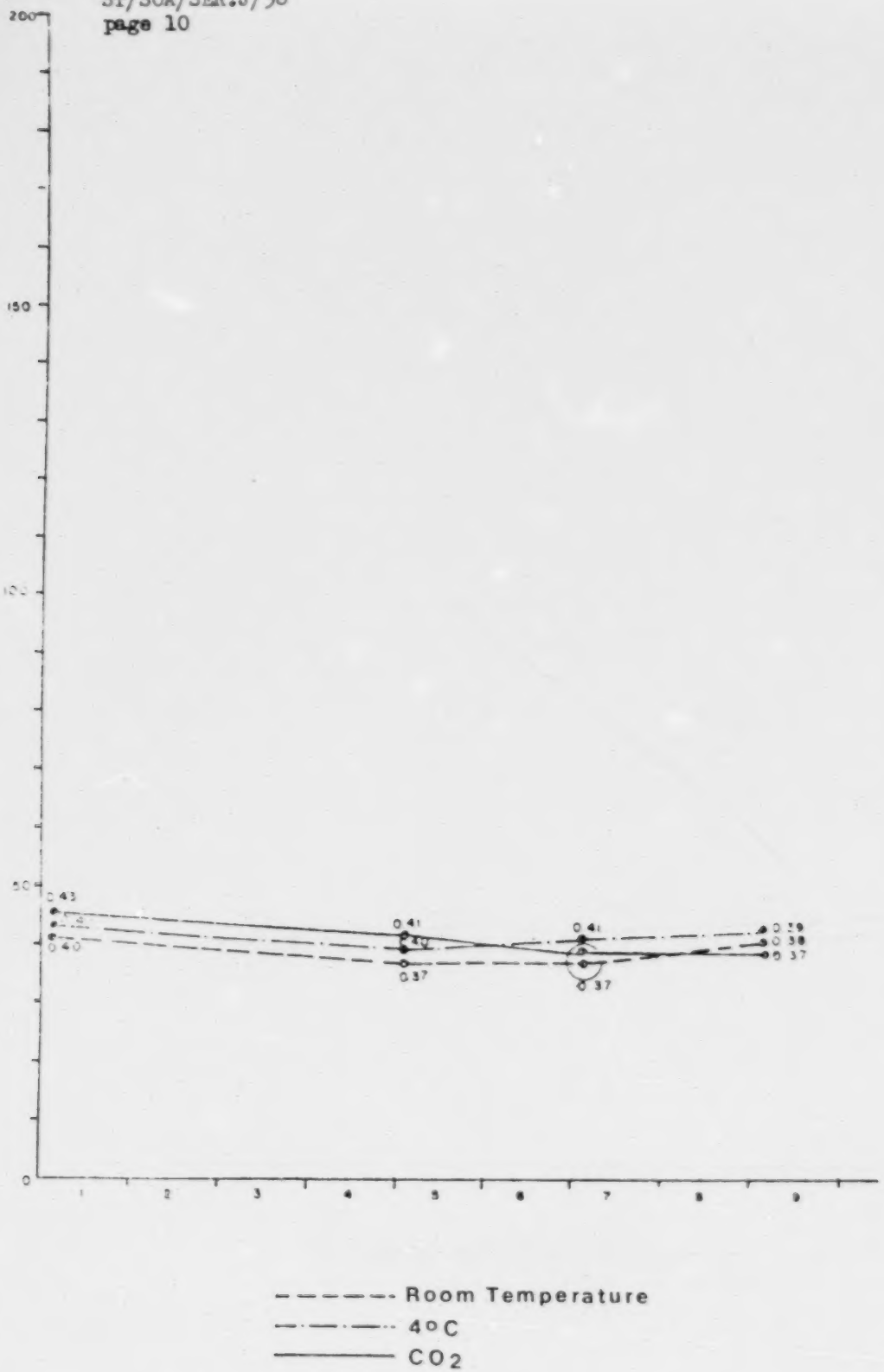


Figure 5 - Changes in morphine content of P. somniferum - UNS 37 when capsules are compacted as "mini-packs" and stored in different environments.

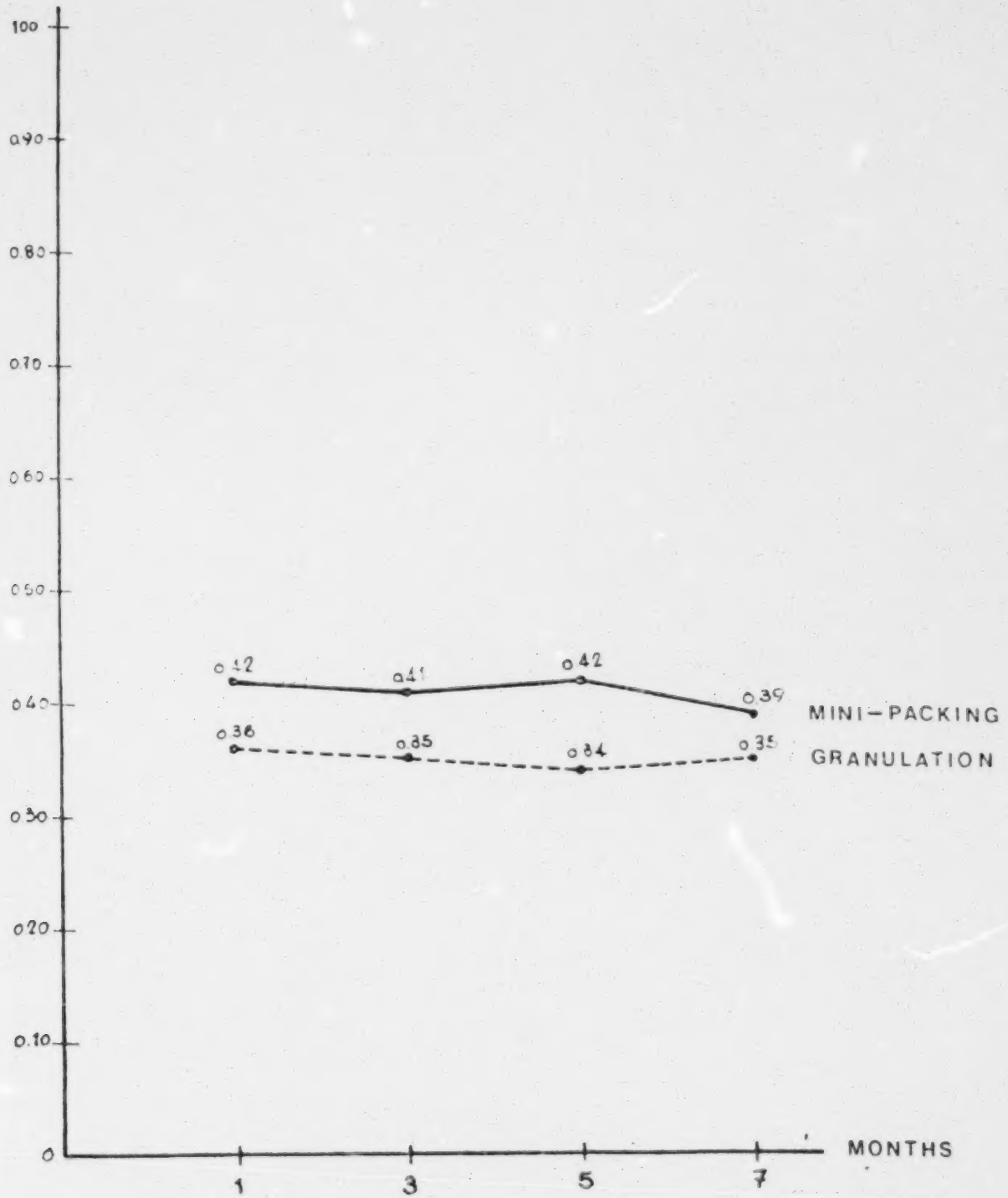


Figure 6 - Comparison of morphine content in poppy straw stored as pulverized powder and compressed "mini-pack".

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/39
14 September 1982

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE[1/]

No. 39

CULTIVATION OF PAPAVER SOMNIFERUM AND PAPAVER BRACTEATUM AND THE QUANTITATIVE DETERMINATION OF SOME ALKALOIDS BY HPLC.

by: K. Wickström, H. Pyysalo and C-J. Widèn
Department of Pharmacy
University of Kuopio
Kuopio, Finland

C.A. Salemink
Organic Chemistry Laboratory
State University
Utrecht, The Netherlands

1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

V.82-30348

INTRODUCTION

Within the scope of our work on the isolation of the major and minor alkaloids of Papaver somniferum L. and Papaver bracteatum Lindl., cultivated under different conditions, it was observed that, as a result of certain spray experiments in the Netherlands, (H.G. Theuns 2/) the amounts of some of the main alkaloids were distinctly increased. However, information was lacking on the amounts of the minor alkaloids, many of which were still unknown at the time of the above-mentioned experiments.

In combination with the research on the isolation and structure elucidation of the minor alkaloids, it was decided to cultivate both Papaver species in different regions in Finland, in order to investigate the possible effects on the alkaloid biosynthesis that might be caused by the accelerated and abundant growth during the short spring and summer period in this northern country.

EXPERIMENTAL

Cultivation:

P. somniferum. The seeds (UNL. nos. 101, 106, 107, 110, 119, 136, 154, 187, 275, 316, 364, 593, 3/) were cultivated in the botanical garden of the University of Kuopio (400 km north of Helsinki), where the soil is clayish and also contains a small amount of peat. Each cultivation consisted of 50 to 100 plants in rows at intervals of 25 cm.

Smaller cultivations were also established in the city area of Helsinki and in the village of Lempäälä, 200 km north of Helsinki. These cultivations which consisted of 10 to 20 plants from each batch of seed were located in normal household gardens with mouldy earth. In Helsinki the plants suffered seriously because of the shade of tall trees.

2/ The results will be published in the forthcoming Ph.D. thesis at the State University of Utrecht, The Netherlands.

3/ The seeds of P. somniferum and P. bracteatum were provided through the courtesy of the UN Narcotics Laboratory.

The seeding time of all the cultivations was 20 May 1979. The first seedlings appeared after two weeks, but the growth of the plants was very slow during the month of June. At the beginning of July a highly intensive growth season began and most plants were in bloom during the last two weeks of July. During the month of July the weather conditions in the Kuopio area were excellent; the weather was sunny, the temperature was on average + 20°C, and adequate rain made the cultivations flourish.

In southern Finland the weather conditions were considerably less favourable and the plants were smaller. In Helsinki the plants reached, on average, one third of the size of the plants in Kuopio.

P. bracteatum. The seeds (UNL. nos. 8, 11, 441, 581 and 594) were cultivated in the botanical garden of the University of Kuopio. The growth during the first year was very satisfactory and healthy plants were obtained. Here too, the seeding took place on 20 May 1979 (for experimental conditions, see those used for Papaver somniferum). About 50 % of the plants survived the hard winter (minimum temperature - 36°C) and, at the end of June, some of the second year plants were flowering. The quantitative determination of the alkaloidal content of the leaves of the first year plants indicated the presence of thebaine and alpinigenine as shown in Table 2.

Sampling:

The plants are described in Table 1. Samples were collected during the growing season at intervals of two weeks. Each time, two plants representing the average stage of development in the cultivation were collected (see Table 1). The plants, including the roots, were rinsed with water. Capsule samples, consisting of five average capsules, were collected twice from each cultivation. Immediately after collection, the samples were dried at + 105°C for two hours and stored at room temperature for 2 to 6 months.

After removal of the seeds, the capsule samples were pulverized (diameter of the particles less than 0.5 mm) with the aid of a Culatti mill. The residual moisture in the material was determined by heating an aliquot just above 100°C until constant weight and the average found (8 %) was taken into account in calculating the alkaloid contents.

500 mg of the powdered sample was suspended in 25 ml of acetic acid (5 %) for four hours. The suspension was filtered and the filtrate was extracted with 20 ml of hexane in order to remove pigments etc. The aqueous phase was made alkaline (pH=9) with the aid of ammonia (25 %) and then extracted three times with 20 ml of a chloroform-isopropanol mixture (3:1). The solvent was evaporated to dryness in vacuo and the residue was dissolved in 0.5 ml of methanol.

HPLC experiments:

The methanol solution (10 µl) was injected into a Waters M 6000 HPLC instrument with a 300 mm x 4 mm Bondapak C_{18} column. The absorption detector was a Waters model 400 equipped with a filter at 280 nm (for all the alkaloids) and at 313 nm (especially for thebaine, noscapine and papaverine).

Several experiments were carried out with various solvent systems and the most suitable system in reverse phase chromatography for separating the alkaloids studied was found to be methanol and water (45 . 55) containing 0.05 M of B5-buffer (1-pentanesulfonic acid sodium salt).

The flow rate was 2.0 ml per minute and the pressure 2000 p.s.i.

The quantitative determinations were based on comparison of the peak areas obtained for the samples with those obtained for the reference substances. A HPLC chromatogram for the standard mixture is presented in figure 1.

DISCUSSION

As shown in Table 1, the intensive growth season in the north produces within a short period of time P. somniferum plants containing considerable amounts of the alkaloids studied. Reference data for earlier cultivations from the same batches of seed were available only for samples UNL 136, 316 and 364.

It seems that in certain plants (UNL 316 and 364) the growing conditions in the north cause the plants to produce even higher amounts of the main alkaloids than in the countries of origin. However, lower concentrations were found as well (UNL 136). Seeds from the same batches, cultivated in different parts of the country, produced varying amounts of the main alkaloids, presumably because of the different growing conditions. The poor growth of the plants in Helsinki produced lower amounts of the main alkaloids. The content of the alkaloids analyzed seems to reach a maximum level at the beginning of August. In this stage of the work, only preliminary first analyses for P. bracteatum were made (Table 3). It is evident that P. bracteatum can be cultivated in southern Finland. However, in the Kuopio area, being only a few hundred kilometers south of the Arctic circle,the hard winter with longer periods of time when the temperature is lower than -20°C, a great proportion of the plants could not survive their first winter.

It is interesting to note (table 3) that the concentration of alpinigenine in one of the plants (UNL 581) almost reaches that of thebaine, the main alkaloid in all of the P. bracteatum samples studied. The cultivation will be continued.

Figure 1. A HPLC chromatogram obtained from a mixture of morphine (1),codeine (2),thebaine (3), noscapine (4) and papaverine (5).

TABLE 1. (data from Kucpio)

UNL number	average height of flowering plant cm	average weight of fresh capsules g	colour of flowers
101	75	9.5	white
106	70	7.5	violet
107	100	12.5	white/violet
110	100	18.0	white
119	50	5.5	pink
136	60	3.7	red/violet
154	30	3.5	white
187	40	3.0	white
275	65	3.5	white
316	30	2.6	white
364	90	2.9	white/violet
593	60	5.8	white/violet

TABLE 2. Alkaloid contents of Papaver somniferum samples

Place of growth: K = Kuopio, H = Helsinki, L = Lempäälä

The data given is the alkaloidal content of the whole plants, including roots, except as indicated in certain cases where the data concerns the capsules only.

UNL data = data on capsules from earlier cultivations of the same batch of seed in the country of origin.

Sample	Date 1979	Place	morphine	codeine	thebaine	noscapine	papaverine
UNL 119	13.7.	K	0.040%	0.005%	0.0028%	0.0065%	0.0040%
"Nauga"	31.7.	K	0.170	0.007	0.0130	0.0087	0.0010
Hungary	26.8.	K	0.099	0.003	0.0036	0.0067	<0.0005
	13.8.	L	0.032	-	<0.0005	0.0045	0.0006
	10.9.	L	0.042	-	<0.0005	0.0045	<0.0005
	29.6.	H	0.020	0.0017	0.0015	0.0007	<0.0005
	25.7.	H	0.037	0.0027	<0.0005	0.0030	0.0010
	29.8.	H	0.060	0.0036	0.0008	0.0062	<0.0005
UNL 107	13.7.	K	0.024	0.0020	0.0008	0.0015	<0.0005
Kék Duna	31.7.	K	0.046	0.0055	0.0090	0.0110	0.0020
Hungary	12.8.	K	0.230	0.0070	0.0060	0.0170	0.0009
	26.8.	K	0.160	0.0190	0.0120	<0.0005	<0.0005
	1.9 (Capsules)	K	0.210	<0.0005	0.0012	0.0120	<0.0005
	13.8.	L	0.025	-	<0.0005	0.0010	<0.0005
	10.9.	L	0.042	-	<0.0005	0.0010	<0.0005
	25.7.	H	0.037	0.0027	<0.0005	0.0030	0.0010
	29.8.	H	0.043	0.0023	<0.0005	0.0042	<0.0005

UNL 136	13.7.	K	0.041	0.0280	0.0055	0.0150	0.0100
Pakistan	31.7.	K	0.060	0.0140	0.0060	0.0290	0.0230
	12.8	K	0.300	0.0200	0.0018	0.1600	0.0300
	12.8. (Capsules)	K	0.380	0.0250	0.0060	0.1400	0.1000
	26.8.	K	0.150	0.0210	0.0025	0.0390	0.0550
	1.9. (Capsules)	K	0.340	0.0190	<0.0005	0.1200	0.0940
		UNL data	1.00	0.09	0.07		
UNL 154	13.7.	K	0.057	0.0100	0.0050	0.0450	0.0095
Afghanistan	31.7.	K	0.063	0.0095	0.0082	0.0210	0.0120
	26.8.	K	0.180	0.0260	0.0200	0.0780	0.0230
	13.8.	L	0.085	0.0130	0.0022	0.0400	0.0105
	10.9.	L	0.048	0.0083	0.0011	0.0400	0.0105
UNL 187	13.7.	K	0.031	0.0140	0.0030	0.0080	<0.0005
Jhalawar	31.7	K	0.061	0.0120	0.0045	0.0180	0.0007
Rajasthan	12.8.	K	0.310	0.0230	0.0140	0.1000	<0.0005
India	26.8.	K	0.220	0.0250	0.0090	0.0730	<0.0005
	12.8. (Capsules)	K	0.390	0.0310	0.0080	0.1600	0.0030
	1.9. (Capsules)	K	0.420	0.0092	0.0005	0.1200	0.0015
	10.9.	L	0.170	0.0190	0.0040	0.1200	0.0145

UNL 101	13.7.		K	0.0240	0.0120	0.0028	0.0054	<0.0005
Turkey	31.7.		K	0.0320	0.0070	0.0040	0.0033	0.0025
	12.8.		K	0.130	0.0210	0.0042	0.0600	0.0100
	26.8.		K	0.120	0.0230	0.0092	0.0140	0.0150
	12.8.	(Capsules)	K	0.330	0.0280	0.0050	0.0650	0.0160
	1.9.	(Capsules)	K	0.200	0.0050	0.0014	0.0180	0.0027
	13.8.		L	0.0530	0.0062	0.0035	-	-.0025
	10.9.		L	0.0230	0.0091	0.0005	0.0110	0.0010
	20.6.		H	0.0150	0.0024	0.0005	0.0043	<0.0005
	29.8.		H	0.028	0.0100	0.0005	0.0160	0.0065
UNL 106	13.7.		K	0.070	0.0130	0.0022	0.0030	<0.0005
Turkey	12.8.		K	0.083	0.0063	0.0015	0.0100	<0.0005
	26.8.		K	0.110	0.0066	0.0025	0.0100	0.0063
	13.8.		L	0.069	0.0030	0.0030	0.0120	0.0007
	10.9.		L	0.029	0.0057	0.0013	0.0110	0.0034
	20.6.		H	0.009	0.0033	0.0011	0.0036	<0.0005
UNL 110	13.7.		K	0.073	0.0120	0.0020	0.0012	0.0007
Australia	12.8.		K	0.530	-	-.0060	0.0280	0.0047
	1.9.	(Capsules)	K	0.430	<0.0005	0.0130	0.0390	<0.0005
	10.9.		L	0.022	0.0013	0.0040	0.0110	<0.0005
	25.7.		H	0.025	0.0013	<0.0005	0.0058	<0.0005
	29.8.		H	0.076	0.0050	0.0011	0.0360	<0.0005

UNL 275	13.7.	K	0.040	0.0060	0.0010	0.0100	0.0006
Aklera	31.7.	K	0.039	0.0088	0.0056	0.0086	0.0015
Rajasthan	31.7.	K	0.067	0.0150	0.0067	0.0130	0.0066
India	12.8.	K	0.300	0.0200	0.0066	0.0760	<0.0005
	26.8.	K	0.230	0.0260	0.0190	0.0870	<0.0005
	12.8. (Capsules)	K	0.350	0.200	0.0080	0.1100	<0.0005
	1.9. (Capsules)	K	0.340	0.0290	0.0030	0.1200	0.0028
	13.8.	L	0.071	0.0077	0.0026	0.0630	0.0008
	10.9.	L	0.079	0.0063	0.0011	0.0460	<0.0005
	20.6.	H	0.016	0.0041	0.0027	0.0019	<0.0005
	25.7.	H	0.058	-	-.0022	0.0190	<0.0005
UNL 316	13.7.	K	0.081	0.0120	0.0038	0.0170	0.0025
Faizabad	31.7.	K	0.120	0.0390	0.0070	0.0230	<0.0005
Uttar	12.8.	K	0.270	0.0270	0.0140	0.0920	<0.0005
Pradesh	26.8.	K	0.250	0.0190	0.0140	0.0850	<0.0005
India		UNL data	0.05	0.09	0.05		
UNL 364	13.7.	K	0.044	0.0180	0.0045	0.0160	0.0010
Rustica 0	31.7.	K	0.120	0.0230	0.0065	0.0210	<0.0005
Spain	12.8.	K	0.300	0.0220	0.0035	0.0820	<0.0005
	26.8.	K	0.240	0.0087	0.0240	0.0500	<0.0005
	1.9. (Capsules)	K	0.480	0.0005	0.0024	0.1300	<0.0005
		UNL data	0.34	0.08	-		

UNL 593	13.7.	K	0.150	0.0240	0.0055	0.0020	0.0135
Australia	31.7.	K	0.076	0.0140	0.0280	0.0015	0.0010
	12.8.	K	0.300	0.0220	0.0035	0.0820	<0.0005
	26.8.	K	0.240	-	0.0350	<0.0005	0.0250
	12.8. (Capsules)	K	0.700	0.0120	0.0260	0.0080	0.0020
	1.9. (Capsules)	K	0.330	0.0220	<0.0005	0.1200	0.0980

TABLE 3. Thebaine and alpinigenine content (% in dry material) in *Papaver bracteatum* plants from the 1st year, grown in Kuopio.

Plants	date	thebaine	alpinigenine
UNL 8	21.7.	0.223	<0.005
	26.8.	0.065	<0.005
UNL 11	26.8.	0.113	<0.005
UNL 594	26.8.	0.210	<0.005
UNL 441	21.7.	0.139	<0.005
	26.8.	0.058	<0.005
UNL 581	21.7.	0.080	0.072
	26.8.	0.138	0.086

UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/40
14 September 1982

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE[1/]

No. 40

QUANTITATIVE DATA ON THE ALKALOID CONTENTS OF PAPAVER SOMNIFERUM AND PAPAVER BRACTEATUM CULTIVATED IN KUOPIO, FINLAND IN 1979 AND 1980

by: K. Wickström and C-J. Widèn
Department of Pharmacy
University of Kuopio
Kuopio, Finland

H. Pyysalo
Department of Chemistry
University of Oulu
Oulu, Finland

C.A. Salemink
Organic Chemistry Laboratory
State University
Utrecht, The Netherlands

1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

V.82-30350

INTRODUCTION

In the previous report on our work (UN document ST/SOA/SER.J/39) concerning the alkaloid content of different samples of Papaver somniferum L. grown in Finland, it appeared that the intensive growth season in northern latitudes produces in a short period of time plants containing considerable amounts of the alkaloids studied, i.e. morphine, codeine, thebaine, noscapine and papaverine. In 1979 when the weather conditions, especially in eastern Finland, were excellent, certain plants such as UNL 136 and 364 produced even higher amounts of alkaloids than in the countries of origin. Likewise, studies on first year leaves of P. bracteatum Lindl. revealed that this species produced thebaine (UNL 581 also produced alpinigenine) as the major alkaloid.

The purpose of the present study was to recultivate UNL 101, 110, 119, 136, 275 and 364 seeds of P. somniferum in 1980 under the same environmental conditions as in Kuopio in 1979, and to find out whether the alkaloid contents are constant or not in different years. In addition, the alkaloid contents of second year leaves and capsules of P. bracteatum grown in the same place were also investigated.

EXPERIMENTAL

P. somniferum seeds (UNL 101, 110, 119, 136, 275 and 364) were cultivated from 20 May in the Botanical Garden of the University of Kuopio (400 km north of Helsinki) in the same way as in 1979. These seeds were supplied through the courtesy of the United Nations Narcotics Laboratory.

The growth and behaviour of the plants were similar to those of the same seeds cultivated in 1979. The summers of 1979 and 1980 were both warm and only slightly rainy in eastern Finland, so there were no major

climatic differences between these years. In both years, flowering of the P. somniferum plants started during the end of July although immature, fully grown capsules were formed in the first half of August. However, the flowering time in 1980 was more irregular than in 1979. For example, some plants were still flowering in October. Distinct differences in the size of the plants and capsules even from the same seeds were observed. The cause for this unusual behaviour is unknown.

As pointed out in our earlier report (UN document ST/SOA/SER.J/39) less than 50 % of the P. bracteatum Lindl. plants survived the hard winter of 1979-80 (minimum temperature -36°C) and the remaining plants died during the summer of 1980. Therefore, only 10 plants were flowering at the end of June and immature capsules were collected on 5 July.

Sampling and analysis

The sampling and HPLC analysis of the materials were carried out in the same way as described in our earlier report. However, only total plants of P. somniferum were analyzed this time.

Results

The morphine, codeine, thebaine, noscapine and papaverine contents of P. somniferum grown in Kuopio in 1980 are presented in Table 1. The concentrations are expressed as percentages of the dry weight of the entire plants (including the roots).

The thebaine and alpinigenine contents of P. bracteatum samples collected in 1979 and 1980 in Kuopio are shown in Table 2.

DISCUSSION

Alkaloids of P. somniferum

Variations in alkaloidal contents

In agreement with the morphological variations, considerable variations in the total and individual alkaloid contents could be observed in the materials of 1979 and 1980. The maximum percentages of most alkaloids were found during the first two weeks of August. Concerning the morphine content, it was found that UNL 364 was rich in morphine both in 1979 and 1980, whereas UNL 110, 136 and 275 were rich in morphine in 1979 but rather poor in 1980. UNL 101 and 119 were poor in morphine in both years. The codeine content of all plants was rather small and regular in 1979, but somewhat higher in 1980, although more irregular values were observed. In 1980, especially relatively high concentrations of this alkaloid were observed in UNL 136, 275, 364 and (less distinct) in 119. In both years lower codeine content values were observed for 101 and 110.

UNL 101 and 110 yielded low concentrations of thebaine both in 1979 and 1980, whereas higher but more irregular values were obtained for 136, 275 and 364. The classification of UNL 119 is more difficult based on the data obtained. The noscapine content was rather irregular. It was generally low both in 1979 and 1980 for UNL 101, 110 and 119 and somewhat higher for UNL 136, 275 and 364. Concerning the papaverine content, UNL 136 was rich in both years, while the rest of the UNL samples were poor both in 1979 and 1980.

There were distinct differences in the contents of the alkaloids studied due to seasonal variation. To give really clear profiles of the alkaloidal contents the sampling should be done at the same optimal developmental stage. In eastern Finland the optimum condidtions seem to be during the first two weeks of August.

Chemoprovarieties

Recent large scale selection studies on different varieties of P. somniferum have revealed that the alkaloid content is distinctly variable in this species (Nyman and Hall 1974, 1976, Nyman and Hansson 1978, Nyman 1978, 1980, a, b). Based on the nomenclature system suggested by Tétényi and Végújfalvi 1965 and the current knowledge of the biosynthetic pathways, Nyman and Hansson 1978 classified their materials in the following way (the alkaloid contents were calculated as percentages on dry, ripe capsules);

1. Chemoconvariety: morphinane (ccv. Morph.)
   A. Chemoprovariety: morphine (cpv. Morph: Mo).
      Only morphinane alkaloids are present in contents <0.03 per cent and morphine is the major alkaloid
   B. Chemoprovariety: thebaine (cpv. Morph: The).
      Only morphinane alkaloids are present in contents <0.03 per cent and thebaine is the major alkaloid
   C. Chemoprovariety: low alkaloid (cpv. Morph: LA).
      Only morphinane alkaloids are present in contents <0.03 per cent and morphine is the major alkaloid

2. Chemoconvariety: isoquinoline (ccv. iQ)
   A. Chemoprovariety: isoquinoline (cpv. iQ)
      Morphinane, benzyl- and phthalideisoquinoline alkaloids are present in contents >0.03 per cent

   B. Chemoprovariety: benzylisoquinoline (cpv. iQ:Benzyl).
      Morphinane and benzylisoquinoline alkaloids are present in contents >0.03 per cent.
   C. Chemoprovariety: phthalideisoquinoline (cpv. iQ:Phthalide).
      Morphinane and phthalideisoquinoline alkaloids are present in contents >0.03 per cent.

Our materials were cultivated under uniform conditions in the Botanical Garden of the University of Kuopio in both years investigated. Therefore all differences in contents of alkaloids must be hereditary, and three different chemoprovarieties can be identified if the material is classified according to the scheme above. As our investigations were made on total dry plants and not on dry capsules, the results are not directly comparable with those of Nyman and Hansson 1978. If this and other methodic differences are neglected, UNL 101, 110 and 119 can be classified as belonging to cpv. Morph: Mo, UNL 136 to cpv. iQ:'Q, and UNL 275 and 364 may be regarded as belonging to cpv. Morph: Phthalide

P. bracteatum

As expected thebaine is always the main alkaloid. However, UNL 581 yielded in addition to thebaine, considerable amounts of alpinigenine. No clear differences between first and second year leaves were found.

Table 1: Alkaloid content of *P. somniferum* - samples grown in Kuopio, Finland in 1980.

Concentration of the alkaloids (% of dry weight)

SAMPLE	DATE	MORPHINE	CODEINE	THEBAINE	NOSCAPINE	PAPAVERINE
UNL 101	28.7.80	0.043	0.0195	0.0011	0.0020	<0.0005
	4.8.80	0.050	0.0193	0.0020	0.0025	<0.0005
	13.8.80	0.045	0.0108	0.0012	0.0015	<0.0005
	26.8.80	0.043	0.0223	0.0016	0.0025	<0.0005
UNL 110	28.7.80	0.158	0.0260	0.0069	0.0397	0.0071
	4.8.80	0.060	0.0041	0.0010	0.0026	<0.0005
	13.8.80	0.042	0.0050	<0.0005	0.0019	<0.0005
	26.8.80	0.091	0.0054	<0.0005	0.0247	<0.0005
UNL 119	28.7.80	0.028	0.0013	0.0009	<0.0005	<0.0005
	4.8.80	0.093	0.0162	0.0048	<0.0005	<0.0005
	13.8.80	0.060	0.0081	0.0010	<0.0005	<0.0005
	26.8.80	0.029	0.0524	0.0219	0.0010	<0.0005
UNL 136	28.7.80	0.051	0.0664	0.0047	0.0279	0.1090
	4.8.80	0.096	0.0313	0.0098	0.0481	0.0610
	13.8.80	0.057	0.0488	0.0034	0.0352	0.0075
	26.8.80	0.096	0.0782	0.0107	0.0239	0.0358

SAMPLE	DATE	MORPHINE	CODEINE	THEBAINE	NOSCAPINE	PAPAVERINE
UNL 275	28.7.80	0.107	0.0426	0.0091	0.0320	<0.0005
	4.8.80	0.140	0.0200	0.0010	0.0640	0.0023
	13.8.80	0.167	0.0792	0.0065	0.0072	0.0055
	26.8.80	0.078	0.0530	0.0094	0.0195	0.0122
UNL 364	28.7.80	0.339	0.0478	0.0072	0.0182	<0.0005
	4.8.80	0.0359	0.0600	0.0151	0.0520	<0.0005
	13.8.80	0.0280	0.0277	0.0054	0.0540	<0.0005
	26.8.80	0.360	0.0250	0.0021	0.0577	<0.0005

Table 2. Alkaloid contents of P. bracteatum samples grown in Kuopio

Samples of first year leaves 1979

Content of alkaloids, % of dry weight

Sample	Date	Thebaine	Alpinigenine
UNL 8	21.7.	0.223	<0.005
	26.8.	0.065	<0.005
UNL 11	26.8.	0.113	<0.005
UNL 441	21.7.	0.139	<0.005
	26.8.	0.058	<0.005
UNL 581	21.7.	0.080	0.072
	26.8.	0.138	0.082
UNL 594	26.8.	0.210	<0.005

Samples of second year leaves and capsules 1980

Content of alkaloids, % of dry weight

Sample	Date	Thebaine	Alpinigenine
UNL 11 leaves	5.7.	0.12	<0.005
UNL 441 leaves	5.7.	0.10	<0.005
UNL 581 leaves	5.7.	0.15	<0.004
caps.	5.7.	0.24	0.09
UNL 594 leaves	5.7.	0.19	<0.005
caps.	5.7.	0.24	<0.005

Literature cited:

Tétényi, P. and Vágújfalvi, D.,
Veränderungen des Alkaloidgehalts von Papaver somniferum L.
2. Mittlg.
Pharmazie 20, 731 (1965).

Nyman, U. and Hall, O.,
Breeding oil poppy (Papaver somniferum) for low content of morphine.
Hereditas 76, 49 (1974).

Nyman, U. and Hall, O.,
Some varieties of Papaver somniferum L. with a changed morphinane alkaloid content.
Hereditas 84, 69 (1976).

Nyman, U. and Hansson, B.,
Morphine content variation in Papaver somniferum L. as affected by the presence of some isoquinoline alkaloids.
Hereditas 88, 17 (1978).

Nyman, U.,
Selection for high thebaine low morphine content (cpv. Morph: The) in Papaver somniferum L.
Hereditas 89, 43 (1978).

Nyman, U.,
Alkaloid content in the F_1 and F_2 generations obtained from crosses between different chemoprovarieties in Papaver somniferum L.
Hereditas 93, 115 (1980).

Nyman, U.,
Selection for high thebaine low morphine content in Papaver somniferum L. II. Studies of the I_4 and I_5 generations of a spontaneous mutant.
Hereditas 93, 121 (1980).

UNITED NATIONS

SECRETARIAT

Distr.
GENERAL

ST/SOA/SER.J/41
25 November 1983
Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER SPECIES

AS SOURCES OF CODEINE, MORPHINE AND THEBAINE */

No. 41

INSECTS INFESTING PAPAVER SPECIES IN SPAIN

By: Octavio Aguar Monterde
General Directorate of Health
General Mola 54
Madrid, Spain

Note by the Secretariat

Opinions expressed in papers in this series are the responsibility of the respective authors and do not necessarily reflect those of the United Nations.

*/ The ST/SOA/SER.J/.. series of documents was previously entitled "Scientific Research on Papaver bracteatum".

V.83-64335

INTRODUCTION

1. With few exceptions, micro-organisms causing infections in plants, once introduced, can be eliminated only by the destruction of the infected host. Consequently, preventive methods and procedures designed to keep plants free of infection are of the utmost importance from the practical point of view.

2. A large number of insects are responsible for transmitting diseases which can eventually destroy entire crops. The causal agents of such diseases are conveyed by insects which introduce them when they penetrate or settle on the plants, or transmit them by means of their mouth parts or ovipositors.

3. The transmitting action of the insects can be classified as persistent or non-persistent, defined as follows:

Persistent: when the insect has to spend a long period on the diseased host in order to acquire the disease-causing micro-organism or when a period of incubation in the insect is required, the micro-organism being subsequently transmitted by the insect after a lengthy period.

Non-persistent: when an insect can pick up the micro-organism (virus, bacteria, fungus) immediately and transmit it forthwith, or within a short period of time, simply by inserting its styli into the susceptible host.

4. The most detailed information possible must therefore be obtained about the disease carriers which attack Papaver if they are to be controlled and the spread of diseases prevented.

INSECTS WHICH ATTACK THE PAPAVER SPECIES

5. In order to carry out a systematic study of the various insects which can cause damage during the development of the poppy, we first drew a distinction between those attacking the capsule and those attacking the rest of the plant.

6. Thus far, we have been able to identify a number of species which attach the capsules before they reach maturity, and another genus which attacks them when they have matured and reached the stage of dehiscence, by entering through the orifices.

7. The insect attacks the immature capsule with its ovipositor, which produces a lesion in the wall of the capsule. If the poppy is of the P. rhoeas species, this results in a circular orifice, while in capsules of the P. somniferum species, it produces an outflow of latex, so that the opening becomes completely blocked and the capsule appears dotted with solid concretions which are simply small quantities of opium. The eggs of the species in question are deposited inside the capsules. We shall endeavour later to trace the life cycle of the insect by studying the larva, during which phase nymphs can be observed at various stages of development.

8. As the capsule matures, these parasites develop within it until they reach the adult stage, although occasionally they are parasitized by other insects within the capsules.

Green capsule	Aulax Pseudotorymus Oryssus
Dry capsule	Oxycarenus

Hymenoptera

Genus Aulax

9. This genus belongs to the family Cynipidae - small and drab insects of which there are some 2,000 known species. In general, the members of this family are herbivorous, creating galls on the infested plants. These galls are so characteristic of each species that, in most cases, it is easier to identify the insect by the plant and the gall than by the imago.

10. The relationship between the plant and the gall-producing insect is still by no means clearly established, particularly as regards why the plant responds to the stimulus provided by the young larva in a very specific way by forming an excrescence which is always of the same type. The species which we found belonging to this family is Aulax papaveris.

Genus Pseudotorymus

11. Another of the insects which we identified as infesting Papaver capsules is a species of this genus belonging to the family Chalcididae.

12. In general, insects belonging to this family are small, and are blue, green or iridescent. Classification is complicated by the large number of insects belonging to the family.

13. In a study of the literature, we were unable to find information on any species of this genus which was a parasite of Papaver. It was therefore tentatively named Pseudotorymus papaveris. The female of the species differs very markedly from the male, being larger, with a more compressed abdomen. The ovipositor is relatively large. This insect appears capable of parasitizing the larvae of other insects.

Genus Oryssus

14. This genus belongs to the family of Oryssidae, which are exceptionally rare insects throughout Europe. The species identified by us is O. gratiosus. In the entomological works consulted, it is not mentioned as infesting Papaver, so that this discovery could be of importance.

15. It is probable that this parasite does not itself infest the capsules and it may be a parasite of one or both of the other two species described above. Consequently, this species could be of interest, not only from the point of view of poppy cultivation, but also for possible use as a biological control, should that prove necessary.

Hemiptera

Genus Oxycarenus

16. These insects belong to the Lygaeidae family and are popularly known as "chinch bugs". They generally feed on the sap of plants. The species which we identified in the genus Papaver - Oxycarenus paliata - can infest only dehiscent species, since it enters the capsules when they have reached

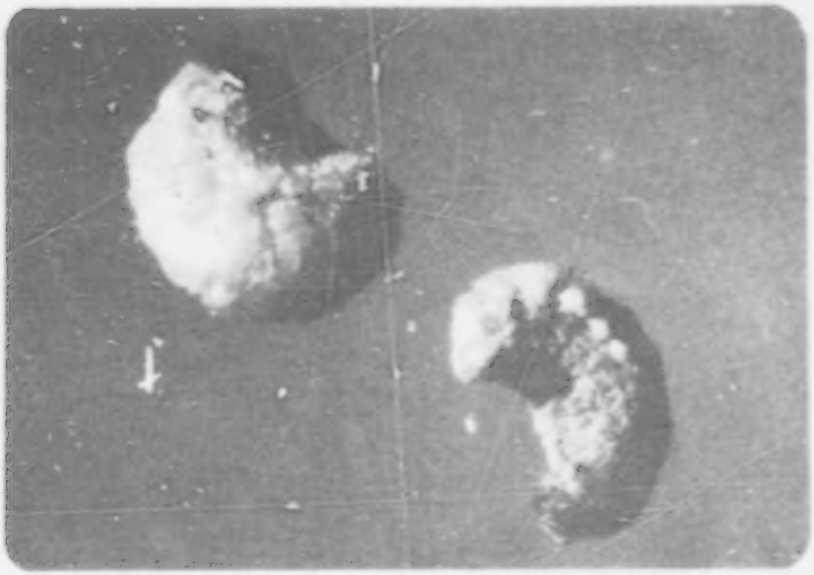
maturity. It enters through the orifices and establishes itself inside the capsule, which it uses as a refuge and place of reproduction, while feeding on the seeds of the plant.

17. We have established that the larvae develop inside the capsule and that the resulting nymphs feed on the seeds: they already have the form of adults, but can easily be distinguished from them by their colouring, which is white with red borders. Inside the capsule, the whole family is protected from attack by arachnids, which are their natural predators.

18. Throughout the winter we have carried out inspections of capsules which have been identified as being infested by O. paliata, finding some specimens in a state of hibernation, which indicates that their life cycle is simple and takes place in one and the same plant.

ILLUSTRATIONS

1. POPPY CAPSULE ATTACKED BY INSECTS



2. AULAX PAPAVERIS LARVAE

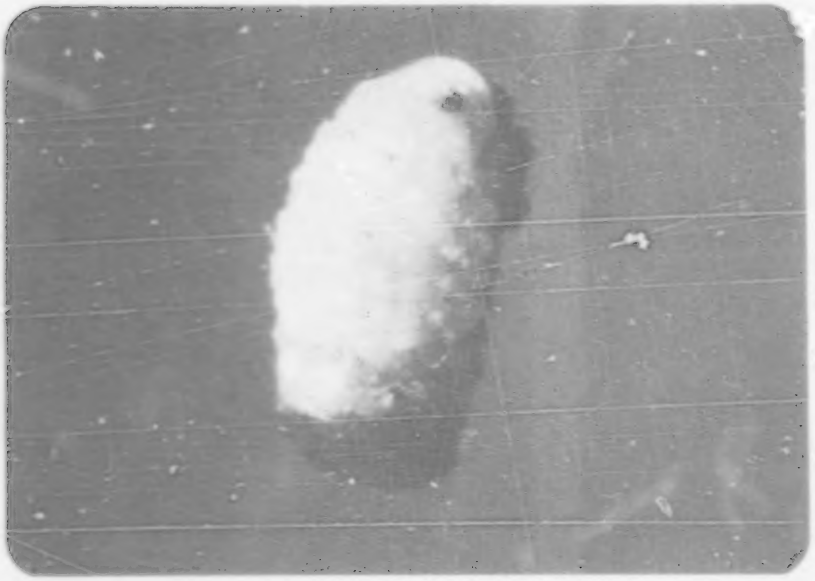
3. AULAX PAPAVERIS NYMPH

4. AULAX PAPAVERIS ADULT

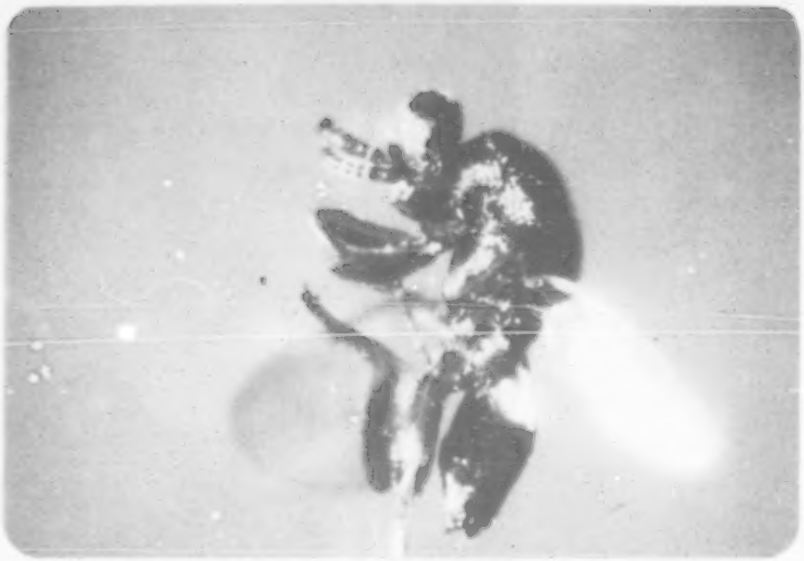
5. AULAX PAPAVERIS ADULT

GENUS PSEUDOTORYMUS

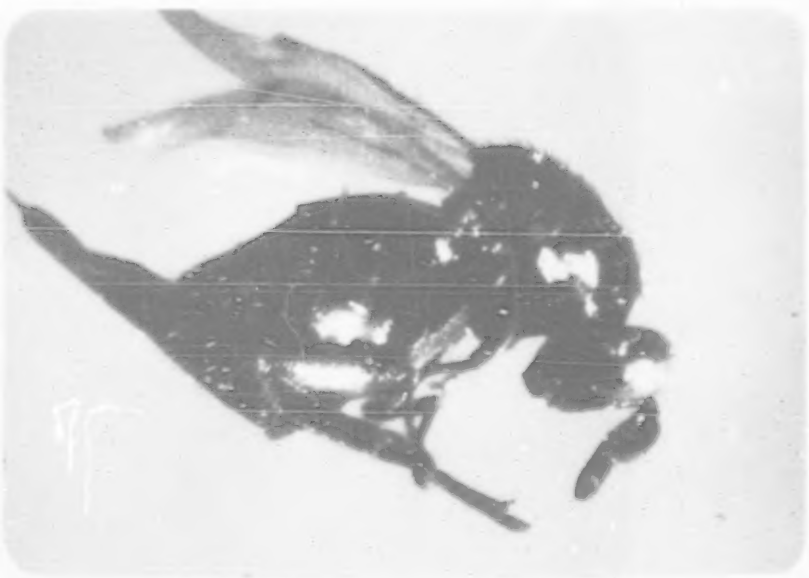
6. PSEUDOTORYMUS PAPAVERIS LARVA



7. PSEUDOTORYMUS PAPAVERIS LARVA



8. PSEUDOTORYMUS PAPAVERIS ADULT



9. PSEUDOTORYMUS PAPAVERIS ADULT

10. PSEUDOTORYMUS PAPAVERIS ADULT

11. PSEUDOTORYMUS PAPAVERIS ADULT

GENUS ORYSSUS

12. ORYSSUS GRATIOSUS ADULT

13. ORYSSUS GRATIOSUS ADULT

GENUS OXYCARENUS

14. OXYCARENUS PALIATA ADULT

15. OXYCARENUS PALIATA ADULT